Biocompatibility of resin-based materials used as pulp-capping agents

C. A. S. Costa[1]**, M. F. Oliveira**[2]**, E. M. A. Giro**[2] **& J. Hebling**[2]

[1]Department of Physiology and Pathology, [2]Department of Orthodontics and Pediatric Dentistry, University of São Paulo State, Unesp, Araraquara, SP, Brazil

Abstract

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Aim To evaluate and compare the response of pulps of rats capped with resin-modified glass-ionomer cement (RMGIC) or self-etching adhesive system.

Methodology Class I cavities were prepared on the occlusal surface of 54 maxillary first molars of 27 rats. Pulp exposure was performed on the cavity floor. The following resin-based materials were applied as pulp-capping agents: G1, Clearfil Liner Bond 2V (CLB 2V; Kuraray Co., Japan); G2, Vitrebond (VIT; 3M/ESPE, USA). In group 3 (control group), a calcium hydroxide/saline paste (CH; Labsynth, Brazil) was used. The cavities were restored with amalgam. After 7, 30 and 60 days, the animals were sacrificed and the jaws were processed for microscopic evaluation.

Results Despite the inflammatory response caused by the experimental and the control materials at 7 days, pulpal healing associated with calcified barrier formation was observed at 60 days following the pulp therapy. Both resin-based materials promoted a large zone of cell-rich fibrodentine matrix deposition on the pulp horn related to the pulp exposure site, which was larger to VIT than to CLB 2V specimens. Tertiary dentine underneath the fibrodentine matrix was deposited by a layer of elongated pulpal cells. The remaining pulpal tissue exhibited normal histological characteristics. In the control group, healing and dentine-bridge formation was observed at 30 days. Pulpal breakdown occurred only when bacterial infection occurred.

Conclusion Both experimental pulp-capping agents allowed pulpal healing characterized by cell-rich fibrodentine and tertiary dentine deposition as well as calcified barrier formation.

Keywords: biocompatibility, bonding agent, glass-ionomer cement, pulp.

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Introduction

Resin-modified glass-ionomer cement (RMGIC) is a specific type of dental material that contains ion-releasing glass particles, water-soluble polyacrylic acids, light-curable monomers and additives (Geurtsen 2000). In contrast to the compomers, the RMGICs exhibit an acid–base reaction with salt formation and a free radical polymerization (Nicholson 1998). Vitrebond (VIT; 3M ESPE, St. Paul, MN, USA) is a light-curing glass ionomer liner/base cement comprised of a powder and a liquid component. This material is widely used in dentistry, being recommended for liner and base applications under composite, amalgam, ceramic as well as cast metal restorations. According to Van Meerbeek *et al.* (1998), bonding agents are fluid resins that can be used in association with restorative materials in order to restore nonretentive defects to natural tooth contour without extensive sacrifice of sound tissue. Despite several *in vitro* studies (Hanks *et al.* 1992, Ratanathien *et al.* 1995, Geurtsen *et al.* 1998, Costa *et al.* 1999) that have demonstrated the cytotoxic effects of different bonding agents and RMGICs, many *in vivo* studies have shown pulpal healing even when resin-based materials were applied as pulp-capping agent (Cox *et al.* 1987, 1998, Akimoto *et al.* 1998). Based upon these *in vivo* studies, it

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Correspondence: Prof. Carlos Alberto de Souza Costa, Department of Physiology and Pathology, University of São Paulo State, Unesp, Rua Humaitá, 1680 – Centro, CEP: 14.801-903/ CP: 331, Araraquara, São Paulo, Brazil (Tel.: +55 16 201 6477; fax: +55 16 201 6488; e-mail: casouzac@foar.unesp.br).

Table 1 Distribution of teeth for experimental and control groups according to the different pulp therapy

Group	Description	Periods (days) 7	30	60	Total
1	Cavity prepare + pulp exposure + CLB 2V primer + CLB 2V bonding + amalgam	5	5	5	15
2	Cavity prepare + pulp exposure + VIT + amalgam	5	5	5	15
3	Cavity prepare + pulp exposure + $Ca(OH)_2$ saline paste + amalgam	5	5	5	15

seems that bacteria and their products rather than the dental materials *per se* play a role in the pulpal breakdown. Consequently, the aim of the present investigation was to evaluate the response of pulps of rats mechanically exposed and capped with two different resin-based materials.

Materials and methods

This *in vivo* study was carried out in accordance with the Guidelines laid down by the US Institutes of Health and the Ethical Committee of the Araraquara School of Dentistry, UNESP, regarding the care and use of animals for experimental procedures.

Fifty-four maxillary first molars of 27 male rats (*Ratus novergicus*, Albinus, Holtzman) weighting approximately 260 g were used. The rats were anaesthetized by intramuscular administration of 10% ketamine (Francotar, Virbac do Brazil Ind. e Com. Ltda., Roseira, SP, Brazil – 0.8 mL per 100 g of body weight) followed by administration of 2% xylazine (Virbaxyl, Virbac do Brazil – 0.04 mL per 100 g of body weight). The animals were placed on a surgical frame (Costa *et al.* 2000b) under aseptic conditions.

After anaesthesia and rubber-dam isolation, the teeth were cleaned with 70% ethanol. Class I cavities were prepared on the occlusal surfaces of the first molars, using a round 0.5 and an inverted cone 33.5 carbide bur at slow speed. Cavities 1.7 mm in depth and 1.3 mm in diameter were washed with copious sterile distilled water to remove debris created during cavity preparation. The pulp tissue was exposed using a sterile sharp right-angled probe. These procedures created a pulp exposure of uniform size. Haemorrhage was controlled with the copious sterile distilled water irrigation.

The pulp exposures were capped with the following experimental or control dental materials: G1, Clearfil Liner Bond 2V (CLB 2V; Kuraray Co., Osaka, Japan); G2, Vitrebond (3M ESPE, St. Paul, MN, USA); and G3 (control group), calcium hydroxide saline paste (CH, Labsynth Produtos para Laboratório, São Paulo, Brazil). The sequence of clinical procedures for applying the experimental and control materials as pulp-capping agents followed the manufacturer's recommendations, which are described in Table 1.

Following application of the experimental materials on the mechanically exposed pulps, the cavities were restored with amalgam. In the control group, a metallic disc was placed on the pulp-capping agent before restoring the cavities. This procedure was performed to prevent direct contact between the restorative and the experimental materials. The animals were kept in specific cages, being fed with regular diet and water *ad libitum*. After 7, 30 and 60 days, the animals were sacrificed.

The maxilla of the animals were surgically dissected *en bloc*, divided in the mid-line, and fixed in 10% buffered formalin solution for 48 h. After demineralization of the maxilla with Morse's solutions for 30 days, the specimens were vacuum-infiltrated with paraffin wax, and finally embedded in paraffin. Six-micrometre-thick serial sections were cut (820 Spencer Microtome, Spencer Products Co., Carson, CA, USA) and stained with haematoxylin and eosin (H&E), Masson's trichrome and Brown & Breen technique for bacterial assessment.

Histological analysis was accomplished using a light microscope (62774, Carl Zeiss Inc., Oberkochen, Germany). As previously described (Costa *et al.* 2000a), four histological features were evaluated, which were graded as shown in Tables 2–5.

Table 2 Inflammatory cell response

Inflammatory cell response	Characterization
Score 0	None or a few scattered inflammatory cells present in the pulp area corresponding to the pulp exposure, characteristic of normal tissue
Score 1	Slight inflammatory cell infiltrate with polymorphonuclears (PMNs) or mononuclear leucocytes (MNLs)
Score 2	Moderate inflammatory cell infiltrate involving the coronal pulp
Score 3	Severe inflammatory cell infiltrate involving the coronal pulp or abscess present

Table 3 Tissue disorganization

Tissue disorganization	Characterization
Score 0	Normal tissue
Score 1	Discrete pulp disorganization close to the pulp exposure site but central pulp normal
Score 2	Moderate disorganization of the pulp tissue morphology
Score 3	Total pulp tissue disorganization or pulp necrosis

Table 4 Hard tissue formation

Reactional dentine formation	Characterization
Score 0	Absence
Score 1	Modest hard tissue deposition beneath or around the exposed area
Score 2	Moderate hard tissue deposition beneath or around the exposed area
Score 3	Intense hard tissue deposition beneath the exposed area characterizing complete dentine bridge

Table 5 Stained bacteria

Bacteria presence	Characterization
Score 0	Absence
Score 1	Presence of stained bacteria along the cavity lateral walls
Score 2	Presence of stained bacteria along the cavity lateral and axial walls
Score 3	Presence of stained bacteria along the cavity walls within the cut dentine tubules or over the pulp tissue

In the present study, the deposition of dentine matrix was reported according to Smith *et al.* (1995) who proposed the following definitions:

Reactionary dentine: dentine matrix secreted and laid down by surviving postmitotic odontoblast cells in response to an appropriate stimulus.

Reparative dentine: dentine matrix secreted and laid down by a new generation of odontoblast-like cells in response to an appropriate stimulus, after the death of the original postmitotic odontoblasts responsible for primary and physiological secondary dentine secretion.

Results

At 7 days, an inflammatory pulpal response with partial tissue necrosis was observed for both experimental and control materials. Disorganization of the pulp tissue and dilated and congested blood vessels associated with lack of dentine-bridge formation were the histological findings frequently observed in most of the specimens. With time, all pulp-capping agents allowed pulp healing and deposition of complete dentine bridges. Pulp reaction graded according to defined criteria is shown in Table 6.

G1 – Clearfil Liner Bond 2V

At 7 days, a necrotic tissue adjacent to the pulp-capping agent was observed. In four specimens, a moderate inflammatory pulpal response mediated by polymorphonuclear cells (neutrophils) occurred in the whole pulpal horn. This pulp response characterized a discrete-to-moderate tissue disorganization (Fig. 1a). In one specimen, pulpoblasts seemed to synthesize fibrodentine matrix (Fig. 1b). In this specimen, primary odontoblasts adjacent to the inflammatory reaction synthesized and deposited a small amount of dentine matrix with cellular inclusions between the proximal and medial pulpal horn. The remaining pulp tissue exhibited a mild inflammatory response associated with a few dilated and congested blood vessels. Proliferation of pulp cells was observed in all specimens of this experimental group. On the other hand, bacteria were not observed in any of the five specimens evaluated.

At 30 days, the small area of necrotic tissue remained adjacent to the pulp-capping agent. Intense deposition of fibrodentine replaced the inflammatory zone observed at 7 days. This fibrodentine matrix exhibited cellular inclusions (Fig. 2a). In two specimens, dentine chips

Table 6 Grading of histological features of sections

Days	Total teeth	Inflammatory response 0	1	2	3	Tissue disorganization 0	1	2	3	Hard tissue 0	1	2	3	Bacteria present 0	1	2	3
CLB 2V (Exp.)																	
7	6	0	1	4	0	0	1	4	0	4	1	0	0	5	0	0	0
30	6	0	4	0	1	0	4	0	1	1	0	2	2	4	0	0	1
60	6	4	0	0	1	4	0	0	1	1	0	0	4	4	0	0	1
VIT (Exp.)																	
7	6	0	0	3	2	0	3	2	0	5	0	0	0	5	0	0	0
30	6	0	4	0	1	0	4	0	1	0	1	4	0	4	0	0	1
60	6	3	0	0	1	3	0	0	2	1	1	0	3	3	0	1	1
CH (control)																	
7	6	0	2	3	0	0	2	3	0	3	2	0	0	5	0	0	0
30	6	3	2	0	0	4	1	0	0	0	0	3	2	5	0	0	0
60	6	3	0	0	2	3	0	0	2	1	1	0	3	3	0	0	2

included in the fibrodentine matrix were observed. Beneath this fibrodentine matrix, a layer of elongated cells deposited reparative dentine matrix that was partially tubular (Fig. 2b) and continuous with the reactionary dentine secreted by primary odontoblasts. Both types of dentine created a calcification barrier. The subjacent pulp tissue exhibited few inflammatory mononuclear cells with small numbers of hyperaemic blood vessels. In one specimen, bacteria were observed on the pulp tissue. In this condition, complete pulp necrosis and a microabscess occurred without formation of a calcified barrier.

At 60 days, the pulp tissue had large areas of fibrodentine matrix adjacent to the pulp-capping material. This fibrodentine associated with an intense deposition of reparative and reactionary dentine gave rise to a thick calcified barrier, which occupied the whole pulpal horn (Fig. 3a,b). The subjacent pulp tissue exhibited normal histological characteristics. Bacteria were observed in one specimen that exhibited pulpal breakdown.

G2 – Vitrebond

At short-term evaluation (7 days), components of Vitrebond displaced into the pulp tissue were observed that caused a large area of pulpal degeneration and necrosis (Fig. 4a,b). The subjacent pulp tissue exhibited a moderate-to-intense inflammatory response related to congested blood vessels and fibroblastic proliferation (Fig. 4b). No dentine matrix deposition was observed. In

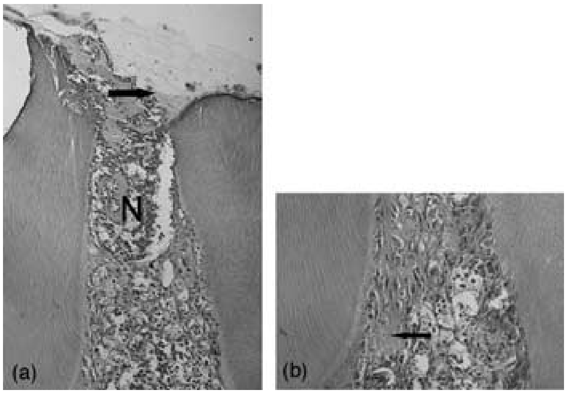

Figure 1 CLB 2V, 7 days. (a) Pulp exposure capped with CLB 2V (arrow). Note that below the necrotic zone (N) there is an intense inflammatory response associated with tissue disorganization (H&E, 56×). (b) Detail of the pulp horn capped with CLB 2V, in which pulpoblasts are depositing fibrodentine (arrow) (H&E, 125×).

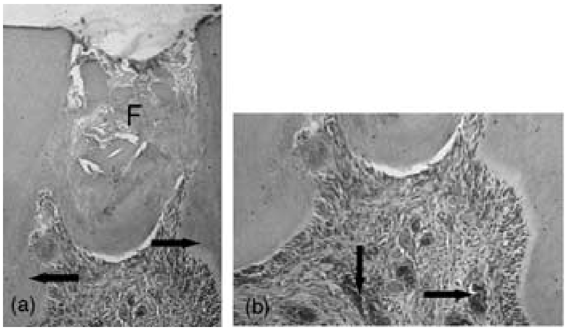

Figure 2 CLB 2V, 30 days. (a) Intense deposition of cell-rich fibrodentine is observed adjacent to the pulp-capping agent (F). Reactionary dentine is also deposited by the primary odontoblast (arrows) (H&E, 56×). (b) Congested blood vessels can be observed in the pulp tissue beneath the fibrodentine matrix (arrows) (H&E, 56×).

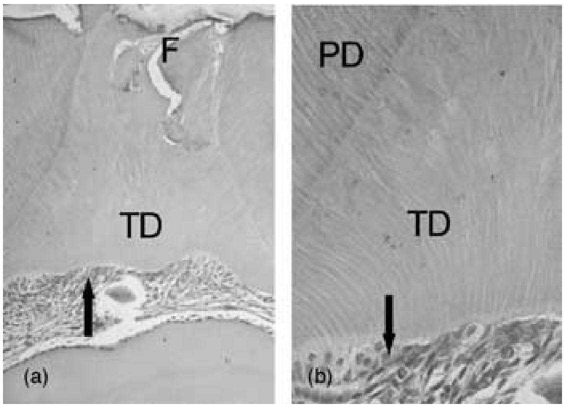

Figure 3 CLB 2V, 60 days. (a) Intense deposition of fibrodentine matrix (F) and tertiary dentine (TD) gave rise to a thick calcification barrier, which occupied the whole pulpal horn capped with CLB 2V. Note a layer of elongated cells (arrow) underlying the dentine matrix (H&E, 56×). (b) High magnification of (a). The original odontoblast cells (arrow) synthesized and deposited the TD that is continuous with the primary dentine (PD) (H&E, 280×).

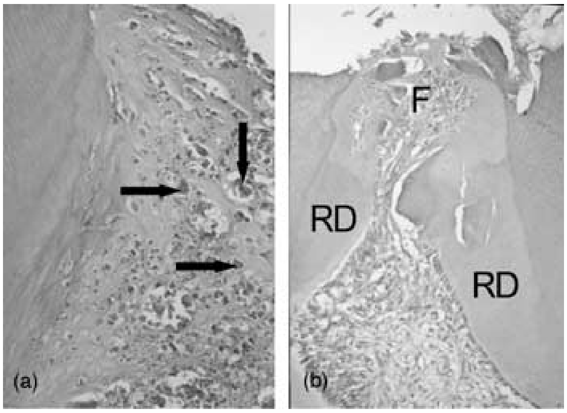

Figure 5 VIT, 30 days. (a) Adjacent to the pulp exposure site capped with VIT, there is an intense deposition of fibrodentine matrix (F). Cell debris and fragments of pulp-capping agent are observed within the fibrodentine (arrows) (H&E, 260×). (b) Note the fibrodentine matrix (F) and lateral reactionary dentine (RD) deposited beneath the pulpal wound (H&E, 56×).

addition, no specimens exhibited bacteria at the cavity walls or into the pulp tissue.

With time (30 days), fibrodentine matrix was observed adjacent to the pulp-capping agent. Components of Vitrebond as well as cellular debris remained inside the fibrodentine matrix (Fig. 5a). Primary odontoblasts synthesized and deposited tubular reactionary dentine underneath the large zone of fibrous reparative matrix. No odontoblast-like cell differentiation was observed adjacent to the fibrodentine. Reactionary dentine continuous with the fibrodentine matrix gave rise to a partial calcified barrier (Fig. 5b). The subjacent pulp tissue exhibited mild mononuclear inflammatory cells. In one specimen, bacteria were observed at the lateral cavity walls. In this specimen, necrosis and zones of microabscesses were observed in the pulp tissue.

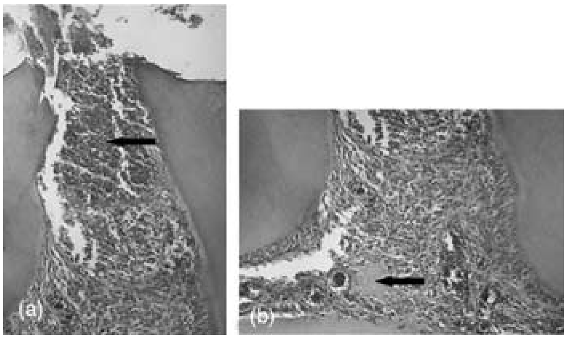

Figure 4 VIT, 7 days. (a) Pulp tissue capped with VIT exhibits a large zone of cell degeneration and necrosis (arrow) (H&E, 64×). (b) A deep zone of the pulp horn capped with VIT. Disruption of the odontoblast layer associated with intense inflammatory response and tissue disorganization can be observed. Note that discrete deposition of dentine matrix can be seen at the base of the pulp horn (arrow) close to the roots (H&E, 125×).

At 60 days, pulpal breakdown was observed in two specimens, in which bacteria were present at the pulp exposure site. In one of these two specimens, periapical lesions associated with root resorption occurred. In three specimens, a thick calcified barrier was observed between the pulp exposure site and the normal pulp tissue (Fig. 6a,b). This calcified barrier was made up of intense deposition of cell-rich fibrodentine matrix continuous with a tubular reactionary and reparative dentine deposited by primary and differentiated odontoblast-like cells, respectively (Fig. 6c,d). A layer of elongated cells was underlying the dentine matrix. The subjacent pulp tissue exhibited normal histological characteristics (Fig. 6d).

G3 – calcium hydroxide paste (control)

At 7 days, a layer of necrosis was observed adjacent to the pulp-capping agent. The pulp tissue subjacent to the necrotic zone exhibited a discrete-to-moderate inflammatory response associated with fibroblastic proliferation. Between the pulp tissue and the necrotic zone

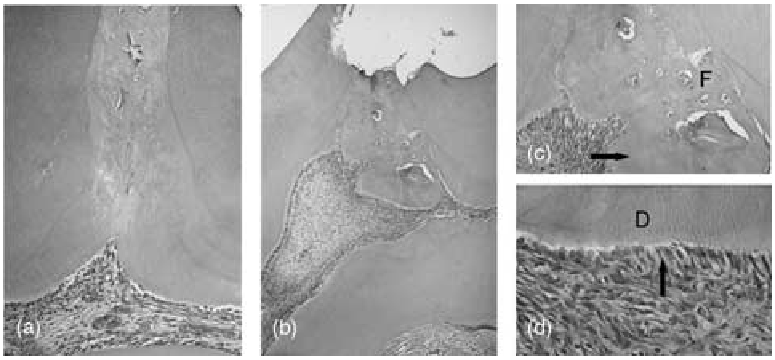

Figure 6 VIT, 60 days. (a) Intense deposition of dentine matrix was observed beneath the pulp exposure site capped with VIT (H&E, 56×). (b) Note, that a large zone of the pulp horn is occupied by dentine matrix (H&E, 32×). (c) High magnification of part (b). The pulp tissue adjacent to the pulp-capping agent exhibits a wide zone of cell-rich fibrodentine (F) associated with tertiary dentine deposition (arrow) (H&E, 56×). (d) Detail of the deepest zone of the calcified barrier. Note the new odontoblast layer (arrow), which secreted and deposited a tubular dentine matrix (d) (H&E, 230×).

pulpal cells deposited a thin and discontinuous layer of dentine matrix, which was clearly demonstrated by Masson's trichrome staining technique (Fig. 7a,b).

At 30 days, differentiation of odontoblast-like cells was observed giving rise to a new layer of elongated cells that synthesized and deposited reparative dentine matrix adjacent to the necrotic zone (Fig. 8). The subjacent pulp tissue exhibited a few scattered mononuclear inflammatory cells and hyperaemic capillaries associated with pulpal cell proliferation. The histological evaluation of the specimens stained with the Brown & Brenn technique did not demonstrate bacteria at the cavity walls.

At 60 days, intense deposition of tubular reparative dentine continuous with the reactionary dentine deposited by primary odontoblasts around the pulp exposure site gave rise to a defined calcified barrier (Fig. 9a). The subjacent pulp tissue exhibited normal histological characteristic (Fig. 9b). Bacteria were observed in two specimens, in which pulpal breakdown occurred (Fig. 10a,b). In one of these specimens, there was deposition of dentine matrix among dentine chips resulting in the formation of an irregular calcified barrier (Fig. 10b).

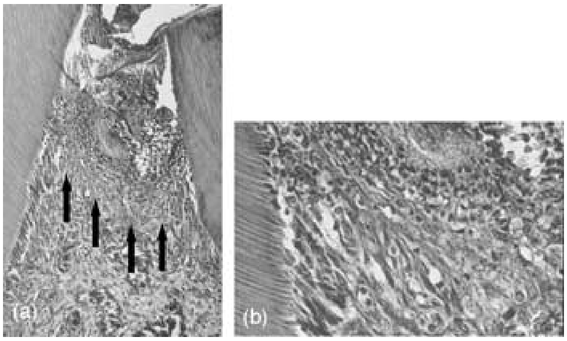

Figure 7 Control group, 7 days. (a) Observe the necrotic tissue adjacent to the pulp exposure site capped with calcium hydroxide. Beneath the necrosis one can see discrete deposition of dentine matrix (arrows). Masson's trichrome, 64×). (b) High magnification of part (a). Note a layer of dentine matrix deposited beneath the necrotic tissue (Masson's trichrome, 230×).

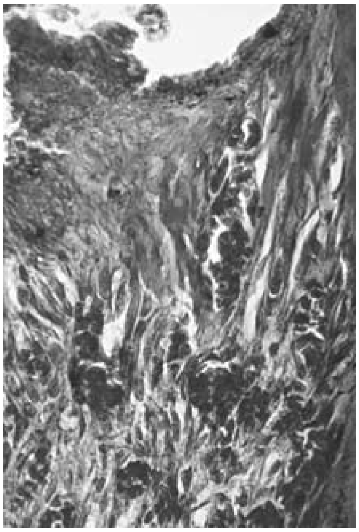

Figure 8 Control group, 30 days. The new odontoblast layer is underlying the calcified barrier, which exhibits cells and blood vessel included. The subjacent pulp tissue shows a number of dilated and congested capillaries (Masson's trichrome, 360×).

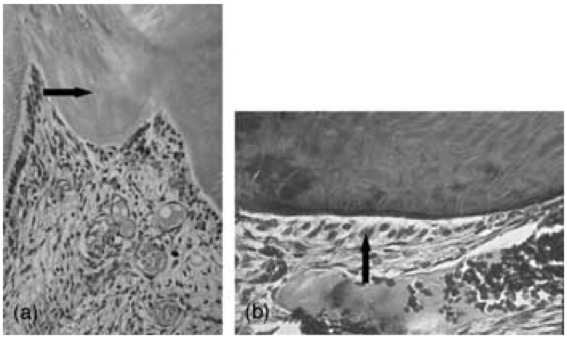

Figure 9 Control group, 60 days. (a) Complete calcified barrier deposited adjacent to the pulpal wound (arrow). Note elongated cells underlying the dentine matrix. The subjacent pulp tissue exhibits normal histological characteristic (H&E, 125 ×). (b) Detail of the deepest zone of the dentine barrier, which is tubular and underlined by a layer of odontoblast-like cells (arrow) (Masson's trichrome, 260 ×).

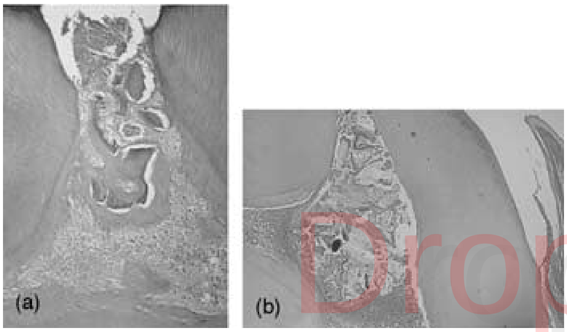

Figure 10 Control group, 60 days. (a) General view of the pulp exposure site. Note the pulp breakdown beneath partial dentine barrier (H&E, 56 ×). (b) Detail of a specimen in, which the restoration was lost during the experiment. Observe presence of debris suggestive of food inside the pulp horn, which exhibits necrosis (H&E, 32 ×).

Discussion

There is controversy concerning the value of using animals for evaluation of the biocompatibility of dental materials. According to Browne (1994), these controversies have arisen for different reasons, such as the methodologies used by different workers have not been standardized. Consequently, outcomes of *in vivo* studies (usage tests) performed in different laboratories have been difficult to interpret. In addition, Browne (1994) reported that usage tests evaluated specifically the biocompatibility of the material rather than the variables that may interfere with the outcome of the test. However, these tests remain the only acceptable manner of testing for the biocompatibility of dental materials.

Different animal species have been recommended for use in pulp or dentine tests in order to evaluate the biocompatibility of experimental dental materials (ISO DIS 7405 1994). Although most of these tests have been carried out in nonhuman primate teeth, many workers have used the teeth of rats or ferrets as experimental models for evaluation of the response of pulps to dental materials (Wennberg *et al.* 1983, Watts & Paterson 1987, Tsuneda *et al.* 1995, Hayashi *et al.* 1999, Costa *et al.* 2000b, Six *et al.* 2000). The relatively high costs of *in vivo* studies using animals such as nonhuman primates have led to the use of small animal species such as rats. This animal model is relatively inexpensive, easier to work, allows the usage tests to be performed under laboratory conditions, and provides preliminary comparative evaluation of the biocompatibility of different dental materials. Consequently, *in vivo* studies performed in teeth of the rats may reduce the number of other animal studies to a minimum. These investigations also avoid exposing human being to potentially harmful agents, such as carcinogens.

In the present investigation, teeth of rats were used to evaluate the response of pulps mechanically exposed and capped with the resin-based materials Clearfil Liner Bond 2V and Vitrebond. Tsuneda *et al.* (1995) evaluated the histological pulp responses of four different bonding agents placed directly on exposed pulp tissue of rats. They reported that one experimental material elicited a slight inflammatory pulpal response initially. With time, this resin-based material allowed pulpal healing associated with moderate deposition of reparative dentine matrix beneath the exposed pulp surface. On the other hand, three experimental materials triggered an intense inflammatory response that appeared to be related to their poor adhesion resulting in microleakage. Tsuneda *et al.* (1995) concluded that these excellent histopathological results observed with a specific bonding agent was probably attributed to its high adhesiveness to the teeth that avoided marginal gap formation and consequently bacterial infection. In accordance with Tsuneda *et al.* (1995), in the present investigation intense inflammatory response associated with pulpal breakdown occurred only when bacteria contamination was observed. The correlation between pulpal breakdown and bacterial contamination has been extensively discussed (Bergenholtz 2000). Costa *et al.* (2000b) evaluated the response of the pulps of rats mechanically exposed and capped with Prime & Bond 2.0 (PB2.0; Caulk/Dentsply, Mildford, DE, USA) bonding agent. The present authors demonstrated that PB2.0 triggered a noticeable inflammatory response initially. However, fibrodentine deposition beneath the pulpal wound was observed to be associated with differentiation of odontoblast-like cells that synthesized and deposited tubular reparative dentine. Similar pulp responses were observed in the present *in vivo* study for both experimental resin-based materials evaluated. However, application of VIT on the pulpal wound caused larger zones of fibrodentine deposition when compared with CLB 2V. It may be speculated that VIT components were displaced further into the pulp tissue (at 7 days) and caused a wide zone of pulp necrosis, in which dead cells and VIT components remained. With time, the fibroblastic proliferation and fibrodentine deposition replaced this large zone of necrosis. On the contrary, discrete displacement of CLB 2V components caused less pulp breakdown that was replaced by fibrodentine, in which small amounts of resin components and pulp cells were included. These histopathological features may be explained by the composition of both experimental resin-based materials.

Geurtsen *et al.* (1998) reported that VIT released decomposition products and additives in aqueous medium that presented a high cytotoxic effect. This was confirmed by Nascimento *et al.* (2000), following application of VIT as a pulp-capping agent in human teeth. These authors demonstrated intense diffusion of VIT components further into the pulp that triggered a wide and persistent inflammatory response mediated by macrophages and small giant cells. These pulp responses did not allow pulp healing or dentine-bridge formation even 300 days after pulp therapy. Displacement of VIT components into the pulp space was also observed in the present investigation. However, rather than a persistent inflammatory reaction, as observed in the human pulp, VIT applied against the pulp of rats allowed fibroblastic proliferation, fibrodentine deposition as well as odontoblast-like cell differentiation. Consequently, at 60 days, complete pulpal healing associated with calcified barrier formation was observed. These histological findings were also shown when CLB 2V was used in the present *in vivo* study as pulp-capping agent.

Costa *et al.* (2001) assessed the response of human pulps capped with CLB 2V bonding agent and demonstrated that this self-etching bonding agent released only small amounts of its components into the pulp space. However, the displaced CLB 2V components seemed to be enough to trigger a persistent inflammatory response mediated by foreign body giant cells and macrophages. This pulpal reaction prevented pulpal healing and dentine-bridge formation. Based upon the differences between the histological findings reported in humans' and rats' teeth, it can be suggested that results from *in vivo* studies performed in animal teeth cannot be directly extrapolated to the human circumstances. This statement has been widely discussed in the dental literature (Wennberg *et al.* 1983, Browne 1994, ISO DIS 7405 1994, Costa *et al.* 2000a). Costa *et al.* (2000a) reported that even the outcomes from *in vivo* studies carried out to evaluate the biocompatibility of dental materials applied to pulp exposures performed in sound human teeth cannot be extrapolated to clinical conditions, especially when the teeth exhibit pulp exposures caused by caries.

Gatti & Rivasi (2002) reported some specific clinical situations, in which human beings presented periodic fever of unclear origin, lymphoadenopathy and hepatosplenomegaly, haematomegaly, microhaematuria and metabolic acidosis. The renal biopsy of the patient's kidney and liver showed nephropathy with interstitial fibro-sclerosis and infiltrates. The histological sections of the granulomatosis reaction of the liver biopsy exhibited particulates up to 20 μm (average about 10 μm) with different chemical composition, such as silicon, oxygen, sodium, magnesium, aluminium, carbon, cobalt–chrome alloy, barium sulphate, chlorine, potassium, sulphur and calcium. The present authors suggested that the liver and kidneys physiologically filter particles that can pass through the intestinal barrier to reach the bloodstream. In the present investigation, the pulp-capping agents were applied directly on blood vessels and pulp cells. We may speculate that part of the displaced dental material observed in the sections stained with haematoxylin and eosin might be carried to the bloodstream to reach different organs. The liver and kidneys of the animals in this *in vivo* study were not evaluated. However, we may suggest that the organs of the animals undergoing pulp therapies should be removed surgically and evaluated in order to determine the possibility of soluble compounds of pulp-capping agents reaching these organs and causing systemic problems.

Conclusion

According to the experimental condition, it was concluded that both experimental pulp-capping agents allowed pulpal healing characterized by cell-rich fibrodentine and tertiary dentine deposition as well as calcified barrier formation.

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